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Commercially produced spray-dried porcine plasma contains increased concentrations of porcine circovirus type 2 DNA but does not transmit porcine circovirus type 2 when fed to naïve pigs

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ABSTRACT: The porcine circovirus type 2 (PCV2) antibody and DNA status of porcine plasma products collected during the commercial spray-drying process were evaluated. Samples evaluated included 52 pooled liquid plasma (fresh) samples collected at 14 regional abattoirs before transport to 1 of 2 spray-drying facilities, 32 pooled liquid plasma (concentrated) samples collected after arrival at the spray-drying facilities at different stages before the spray-drying process, and 32 samples in powdered form (spray-dried) collected after spray drying. All 116 samples were positive for PCV2 antibody, with PCV2 ELISA sample-to-positive ratios ranging from 9.2 to 13.6 on a DM basis. Porcine circovirus type 2 DNA (4.5 to 7.9 log₁₀ PCV2 copies/mL, DM basis) was present in 82.7% (43/52) of the fresh plasma samples, 71.9% (23/32) of the concentrated plasma samples and 78.1% (25/32) of the spray-dried plasma samples, with a greater prevalence of PCV2b than PCV2a. To determine the infectivity of PCV2

DNA-positive commercial spray-dried plasma, nine 10-wk-old 68-kg PCV2-naïve pigs were randomly assigned to 1 of 3 treatment groups and rooms: 1) a negative control (no plasma in the feed, not inoculated with PCV2); 2) a positive control (no plasma in the feed, inoculated with PCV2); and 3) plasma-fed pigs (4% porcine plasma in the feed for 42 d, not inoculated with PCV2). All positive control pigs became viremic by 7 d postinoculation and seroconverted by 42 d postinoculation, whereas pigs in the negative control group and in the spray-dried plasma group were PCV2 PCR negative and did not seroconvert to PCV2 for the duration of the study. The results indicate that PCV2 DNA and antibodies are commonly found in commercial spray-dried plasma. However, no evidence of infectivity of the PCV2 DNA was found in naïve pigs when commercial spray-dried plasma was included in the diet under the conditions of this study.

Key words: porcine circovirus type 2, porcine circovirus type 2 antibody, porcine circovirus type 2 DNA, porcine circovirus type 2 horizontal transmission, spray-dried porcine plasma

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INTRODUCTION

Porcine circovirus (PCV) type 2 (PCV2), a small, circular, nonenveloped, single-stranded DNA virus (Tischer et al., 1982), is the etiological agent of PCV-associated disease (PCVAD; Opriessnig et al., 2007). Serological surveys have shown that most herds worldwide are infected with PCV2 (Allan and Ellis, 2000; Walker et al., 2000; Zhou et al., 2006). Subclinical PCV2 infections are common in pig populations, and

only a small percentage of pigs develop clinical PCVAD (Calsamiglia et al., 2002; Segalés and Domingo, 2002). Porcine circovirus type 2 can be transmitted horizontally by shedding via the oronasal, fecal, and urinary routes (Magar et al., 2000; Bolin et al., 2001; Shibata et al., 2003; Yang et al., 2003) or can be transmitted vertically (Laroche et al., 2000; Kim et al., 2001; McIntosh et al., 2006; Madson et al., 2008).

Spray-dried porcine plasma is commonly included in diets for weanling pigs (Coffey and Cromwell, 2001; Van Dijk et al., 2001; USDA, 2008). Porcine circovirus type 2 is resistant to dry-heat treatment (120°C for 30 min; Welch et al., 2006) and wet-heat treatment (70°C for 15 min; Allan et al., 1994; O'Dea et al., 2008). Therefore, when considering the widespread nature of

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the infection, it is important to determine if spray-dried porcine plasma represents a significant vector for the spread of PCV2 infection. Recently, Patterson et al. (2010) reported that experimentally produced spray-dried porcine plasma, collected using a benchtop laboratory spray drier from a single pig infected with PCV2 exhibiting clinically manifest PCVAD, was capable of transmitting PCV2 to naïve pigs via intraperitoneal inoculation or oral gavage.

The purpose of the current study was to provide information on the prevalence of PCV2 DNA and anti-PCV2 antibodies in commercially produced porcine plasma at different stages of production and to further investigate the potential transmission of PCV2 when commercially produced porcine plasma was included in the diets of naïve pigs.

MATERIALS AND METHODS

The experimental protocol in this study was approved by the Iowa State University Institutional Animal Care and Use Committee.

Spray-Dried Plasma Survey

Overview of the Spray-Drying Production Process. During slaughter at 14 federally inspected abattoirs located in the United States, plasma is collected from each pig and pooled at each individual facility. On a daily basis, the plasma is collected from the abattoirs and transported in insulated tankers to 2 spray-drying facilities located in the Southeast and Midwest areas of the United States. One facility receives plasma from 6 of the abattoirs located in the eastern one-half of the United States, whereas the other facility receives plasma from 9 of the abattoirs located in the Midwest (1 abattoir supplies plasma to both facilities). A tanker contains plasma from approximately 9,000 to 10,000 pigs.

Sample Collection, Storage, and Transport. Pooled liquid plasma (8% solids; $n = 52$) from each of the 14 federally inspected plants was collected from the tankers on arrival at the spray-drying facility in 50-mL conical tubes on 4 different days at different time points.

At the spray-drying facility, plasma from 1 to 3 tankers was combined in a holding silo and scheduled for concentration and spray drying according to the normal production protocol. Samples of both concentrated liquid (28 to 30% solids; $n = 32$) and dried (91 to 93% solids; $n = 32$) plasma were collected at 4 different time points throughout the production cycle. To adequately characterize each production run, samples were taken after initial stabilization of the spray-drying process at the start of each run (beginning), after approximately one-third of the production run (middle 1), after approximately two-thirds of the production run (middle 2), and just before the spray dryer shut down (end of the production run).

All plasma samples were immediately frozen after collection and stored at -20°C . Samples were shipped frozen overnight on wet ice to the analytical laboratory, where they were immediately stored at -80°C until testing.

PCV2 Serology. The presence and quantity of anti-PCV2 antibodies were determined using an open-reading frame 2-based PCV2 IgG ELISA (Nawagitgul et al., 2002). Before detection, spray-dried samples were diluted at 0.167 g/mL in normal saline solution and then further diluted to constant solids of 8% before detection. Pooled liquid plasma and concentrated plasma samples were also diluted to constant solids of 8% before testing. The sample-to-positive (S/P) ratio was calculated by dividing the sample optical density at 450 nm by the positive control optical density, and samples with an S/P ratio of 0.2 or greater were considered positive.

PCV2 PCR. Deoxyribonucleic acid was extracted and immediately frozen at -20°C by using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). The primers and probes designed for PCV2 open-reading frame 1 were used for quantitative real-time PCR (Opriessnig et al., 2003). The real-time PCR reaction consisted of a 25- μL PCR mixture containing 12.5 μL of a commercially available master mix (TaqMan Universal PCR master mix, Applied Biosystems, Carlsbad, CA), 2.5 μL of DNA, 1 μL (0.4 μmol) of each primer, and 0.5 μL (0.2 μmol) of probe. The reaction was run in a 7500 Fast Real-Time PCR system (Applied Biosystems) under the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Serial dilutions of a recombinant PCV2 DNA clone were included in each plate to generate a standard curve. Viral concentrations were expressed as the viral DNA copy numbers per milliliter of sample.

For PCV2a/b open-reading frame 2 differential PCR, a forward (5'-GCAGGGCCAGAATTCAACC-3') and a reverse primer (5'-GGCGGTGGACATGATGAGA-3'), a probe specific for PCV2a (5'-Cal Fluor Orange 560-GGGGACCAACAAAATCTCTATACCCTTT-BHQ-3'), and a probe specific for PCV2b (5'-Quasar 670-CTCAAACCCCCGCTCTGTGCCC-BHQ-3') were designed in the Cap-coding region of PCV2. The multiplex real-time PCR reaction consisted of a total volume of 25 μL containing 12.5 μL of the commercially available master mix (Applied Biosystems), 5 μL of DNA, 0.4 μmol of each primer, and 0.2 μmol of each of the probes. The reactions were carried out under the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The sensitivity and specificity of the real-time PCR reaction were evaluated by using known PCV2a and PCV2b isolates as well as porcine parvovirus, porcine reproductive and respiratory syndrome virus (PRRSV), and PCV1 isolates.

Statistical Analysis. Experimental data were analyzed for each region (Southeast or Midwest) by ANOVA, using PROC GLM (SAS Inst. Inc., Cary, NC).

Least squares means are reported for abattoir samples. A *P*-value of less than 0.05 was considered statistically significant throughout this study. Real-time PCR results (copies/mL of plasma) were \log_{10} transformed before statistical analysis. Because of the different solids concentrations of abattoir samples, all samples were transformed to a DM basis before analysis and are reported in the tables on a DM basis. A χ^2 analysis was used to analyze differences in prevalence.

Bioassay

Animals and Housing. Colostrum-fed, crossbred, specific-pathogen-free, 10-wk-old pigs (approximately 68 kg of BW) were purchased from a herd that is routinely tested for major swine pathogens and known to be free of PCV2, PRRSV, porcine parvovirus, and swine influenza virus. Pigs were housed individually in separate rooms. Each room had 18 m² of solid concrete floor space, separate ventilation systems, and 1 nipple drinker.

Experimental Design and Treatments. In a completely randomized design, 9 pigs were randomly divided into 3 treatment groups, which included 1) a negative control (no plasma in the feed, not inoculated with PCV2); 2) a positive control (no plasma in the feed, inoculated with PCV2); and 3) plasma-fed pigs (4% porcine plasma in the feed for 42 d, not inoculated with PCV2). The diets in treatments 1 and 2 did not contain any animal protein except for dried whey. All diets were formulated to meet the nutritional requirements of the pig (NRC, 1998) and were provided on the floor to ensure ad libitum consumption. Pigs in treatment 2 were inoculated intranasally with 1.5 mL of PCV2b isolate NC-16845 at a tissue culture infective dose (**TCID₅₀**) of $10^{4.5}$ /mL. Blood samples (approximately 5 mL) were collected in serum separator tubes (Fisher Scientific, Pittsburgh, PA) on arrival, on the day of inoculation, and weekly thereafter for 42 d. Blood was centrifuged at $2,000 \times g$ for 10 min at 4°C and tested for the presence of anti-PCV2-IgG (described in the PCV2 Serology section) antibodies and PCV2 DNA (described in the PCV2 PCR section).

Spray-Dried Plasma. The commercially produced spray-dried plasma used in this trial was selected from 2 lots containing increased PCV2 DNA loads (6.7 and 6.4 \log_{10} copies/g, respectively), and ELISA S/P ratios of 1.214 and 1.034 in a dilution of 0.25 g/mL. The 2 lots of plasma were blended 50:50 and then incorporated into the complete feed, for a final inclusion of 4%. Based on average intake of 2.57 kg/d for a 65-kg pig fed for 42 d, average plasma consumption would be 103 g/d, for a total consumption of 4.33 kg of spray-dried plasma per pig.

Necropsy. All pigs were humanely euthanized by pentobarbital overdose (Fatal-Plus, Vortech Pharmaceuticals Ltd., Dearborn, MI) and necropsied at 42 d postinoculation (**dpi**). The severity of macroscopic lung

lesions (ranging from 0 to 100%) was estimated in a blinded fashion as described previously (Opriessnig et al., 2006). Sections of the lymph nodes (superficial inguinal, mediastinal, tracheobronchial, mesenteric), tonsils, thymus, ileum, kidneys, colon, spleen, and liver were collected at necropsy and fixed in 10% neutral-buffered formalin and routinely processed for histological examination.

Histopathology. Microscopic lesions were evaluated by a veterinary pathologist blinded to treatment groups. Lung sections were scored for the presence and severity of interstitial pneumonia, ranging from 0 (normal) to 6 (severe diffuse; Halbur et al., 1995). Sections of the heart, liver, kidneys, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored from 0 (none) to 3 (severe). Lymphoid tissues, including the lymph nodes, tonsils, and spleen, were evaluated for the presence of lymphoid depletion, ranging from 0 (normal) to 3 (severe), and for histiocytic inflammation and the replacement of follicles, ranging from 0 (normal) to 3 (severe; Opriessnig et al., 2004).

PCV2 Immunohistochemistry. Porcine circovirus type 2-specific antigen detection was done by immunohistochemistry on selected formalin-fixed and paraffin-embedded sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), tonsils, spleen, and thymus by using a rabbit polyclonal antiserum (Sorden et al., 1999). The amount of PCV2 antigen was scored by a pathologist blinded to treatment groups. Scores ranged from 0 (no signal) to 3 (more than 50% of lymphoid follicles contained cells with PCV2 antigen staining; Opriessnig et al., 2004).

Statistical Analysis. Analysis of variance was used to determine significant differences among groups. Real-time PCR results (copy numbers) were \log_{10} transformed before statistical analysis. If an ANOVA result was significant (*P* < 0.05), Student's *t*-tests were used to determine which groups were different. Statistical analysis of the data was performed with JMP software (SAS Inst. Inc.).

RESULTS

Spray-Dried Plasma Survey

PCV2 Antibody Status in Different Sample Types and Regions. Fresh, concentrated liquid, and spray-dried plasma samples from within and across the Midwest or Southeast regions were compared (Tables 1 and 2). All fresh (52/52), concentrated liquid (32/32), and spray-dried (32/32) plasma samples contained antibodies to PCV2, with mean ELISA S/P ratios (\pm SEM) on a DM basis of 12.9 ± 0.4 , 12.0 ± 0.7 , and 10.3 ± 0.3 , respectively. By region, ELISA S/P ratios (DM basis) in the Midwest were less (*P* < 0.05) in concentrated liquid compared with fresh plasma samples, whereas the ratio in the spray-dried samples was intermediate. In

Table 1. Porcine circovirus type 2 (PCV2) antibody and DNA copies of fresh, concentrated liquid, and spray-dried plasma samples from a Midwest slaughter region

Sample type	Fresh	Concentrated liquid	Spray dried	SEM
No. of samples	24	12	12	
PCV2 ELISA ¹				
S/P ratio ²	12.44 ^a	9.16 ^b	11.44 ^{ab}	0.92
Prevalence, %	100	100	100	0
PCV2 DNA ¹				
Log ₁₀ copies/mL	7.92 ^a	6.45 ^b	7.76 ^a	0.35
Prevalence, %	100	91.7	100	4.12

^{a,b}Within a row, means without a common superscript differ ($P < 0.03$).

¹Porcine circovirus type 2 ELISA and DNA expressed on a DM basis.

²Sample-to-positive ratio.

the Southeast, ELISA S/P ratios (DM basis) decreased ($P < 0.05$) from fresh to spray-dried samples. Overall, samples from the Southeast region had a greater antibody content for PCV2 compared with those in the Midwest.

PCV2 DNA Status in Different Sample Types and Regions. In total, PCV2 DNA was present in 82.7% (43/52) of the fresh plasma samples, with a mean log₁₀ PCV2 DNA load of 6.5 ± 0.4 on a DM basis. Porcine circovirus type 2a and PCV2b were detected in 25% (13/52) and 82.7% (43/52) of the samples, respectively. Porcine circovirus type 2 DNA was detected in 71.9% (23/32) of the concentrated liquid plasma samples, with a mean log₁₀ PCV2 DNA load of 5.3 ± 0.6 on a DM basis. Porcine circovirus type 2a was detected in 3.1% (1/32) of the concentrated plasma samples, whereas PCV2b was detected in 71.9% (23/32) of the samples. Twenty-five of the 32 (78.1%) spray-dried plasma samples were positive for PCV2 DNA, with a mean log₁₀ PCV2 DNA load of 5.9 ± 0.6 on a DM basis. Porcine circovirus type 2a and PCV2b were detected in 28.1% (9/32) and 71.9% (23/32) of the spray-dried plasma samples, respectively.

Within region, the prevalence of PCV2 DNA in fresh plasma samples was not different ($P > 0.05$); however, the Midwest had a greater prevalence than the Southeast, with a 100.0% prevalence compared with a 67.9% prevalence (Tables 1 and 2). Concentrated liquid plasma samples from the Midwest had a significantly

($P < 0.03$) smaller mean log₁₀ PCV2 DNA load compared with fresh or spray-dried plasma samples (Table 1), which may be the result of a decreased incidence. No difference ($P > 0.05$) in the mean log₁₀ PCV2 DNA load was noted in the Southeast among different sample types (Table 2).

Comparison of In-Process Liquid and Spray-Dried Plasma Samples at Different Stages of Production. Concentrated liquid and spray-dried plasma samples collected from different production stages (beginning, middle 1, middle 2, and end) were compared for their PCV2 antibody and DNA status (Table 3). During the production run, there was no significant change ($P > 0.05$) in the quantity or prevalence of anti-PCV2 antibodies or the load of PCV2 DNA.

Bioassay

Clinical Presentation, Gross and Microscopic Lesions. Clinical disease was not observed in any of the treatment groups. No gross lung lesions were observed in the negative control pigs or the pigs fed spray-dried plasma. Two of 3 positive control pigs had mild gross lung lesions (mean score of 3.3 ± 2.4), characterized by multifocal red to purple consolidation.

Microscopically, none of the negative control pigs had lesions, 1 of the 3 pigs fed spray-dried plasma had mild lymphoid depletion in the tonsils and lymph nodes, and 2 of the 3 positive control pigs had mild to severe lym-

Table 2. Porcine circovirus type 2 (PCV2) antibody and DNA copies of fresh, concentrated liquid, and spray-dried plasma samples from a Southeast slaughter region

Sample type	Fresh	Concentrated liquid	Spray dried	SEM
No. of samples	28	20	20	
PCV2 ELISA ¹				
S/P ratio ²	13.32 ^b	13.66 ^b	9.63 ^a	0.58
Prevalence, %	100	100	100	0
PCV2 DNA ¹				
Log ₁₀ copies/mL	5.22	4.56	4.76	0.83
Prevalence, %	67.9	60	65	10.9

^{a,b}Within a row, means without a common superscript differ ($P = 0.0001$).

¹Porcine circovirus type 2 ELISA and DNA expressed on a DM basis.

²Sample-to-positive ratio.

Table 3. Prevalence and amount of porcine circovirus type 2 (PCV2) antibody and DNA in concentrated and spray-dried plasma samples at different stages of production (mean \pm SEM)

Plasma type	Stage ¹	PCV2 ELISA ²		PCV2 DNA ²	
		Prevalence, No./No. (%)	S/P ratio ³	Prevalence, No./No. (%)	Log ₁₀ copies/mL
Concentrated liquid	Beginning	8/8 (100)	12.8 \pm 1.6	6/8 (75.0)	5.5 \pm 1.2
	Middle 1	8/8 (100)	12.6 \pm 1.3	5/8 (62.5)	4.8 \pm 1.4
	Middle 2	8/8 (100)	13.1 \pm 1.3	6/8 (75.0)	5.4 \pm 1.2
	End	8/8 (100)	9.4 \pm 1.2	6/8 (75.0)	5.4 \pm 1.2
Spray dried	Beginning	8/8 (100)	10.3 \pm 0.9	7/8 (87.5)	6.6 \pm 1.0
	Middle 1	8/8 (100)	10.5 \pm 0.6	5/8 (62.5)	4.8 \pm 1.4
	Middle 2	8/8 (100)	10.6 \pm 0.5	7/8 (87.5)	6.5 \pm 1.0
	End	8/8 (100)	9.8 \pm 0.7	6/8 (75.0)	5.6 \pm 1.2

¹Samples were taken after initial stabilization of the spray-drying process at the start of each run (beginning), after approximately one-third of the production run (middle 1), after approximately two-thirds of the production run (middle 2), and just before the spray dryer shut down (end of the production run).

²Porcine circovirus type 2 ELISA and DNA expressed on a DM basis.

³Sample-to-positive ratio.

phoid depletion and histiocytic replacement of follicles in the tonsils, spleen, and lymph nodes.

Anti-PCV2-IgG Antibody Levels. On arrival, all pigs were negative for the PCV2 antibody (Figure 1). The negative control pigs and pigs fed spray-dried plasma remained negative for the anti-PCV2 antibody throughout the study. Two of 3 positive control pigs were seropositive for PCV2 at 28 and 35 dpi, and by 42 dpi, all 3 pigs had seroconverted to PCV2.

PCV2 Viremia and Antigen in Tissues. All serum samples collected at the beginning of the trial were negative for PCV2 DNA. Negative control pigs and pigs fed spray-dried plasma remained negative for PCV2 DNA in the serum throughout the study (Figure 1). After inoculation, all positive control pigs be-

came PCV2 viremic by 7 dpi and remained PCV2 PCR positive until the termination of the study (Figure 1), indicating that these animals were highly susceptible to PCV2. Porcine circovirus type 2 antigen was not detected in any of the negative control pigs or pigs fed spray-dried plasma. One of 3 positive control pigs had low amounts of PCV2 antigen in the tonsils.

DISCUSSION

The prevalence of PCV2 in subclinically infected slaughter pigs is common (Segalés and Domingo, 2002; Opriessnig et al., 2007). Porcine circovirus type 2 is a stable virus resistant to heat treatment (Welch et al., 2006). Spray-dried porcine plasma collected from

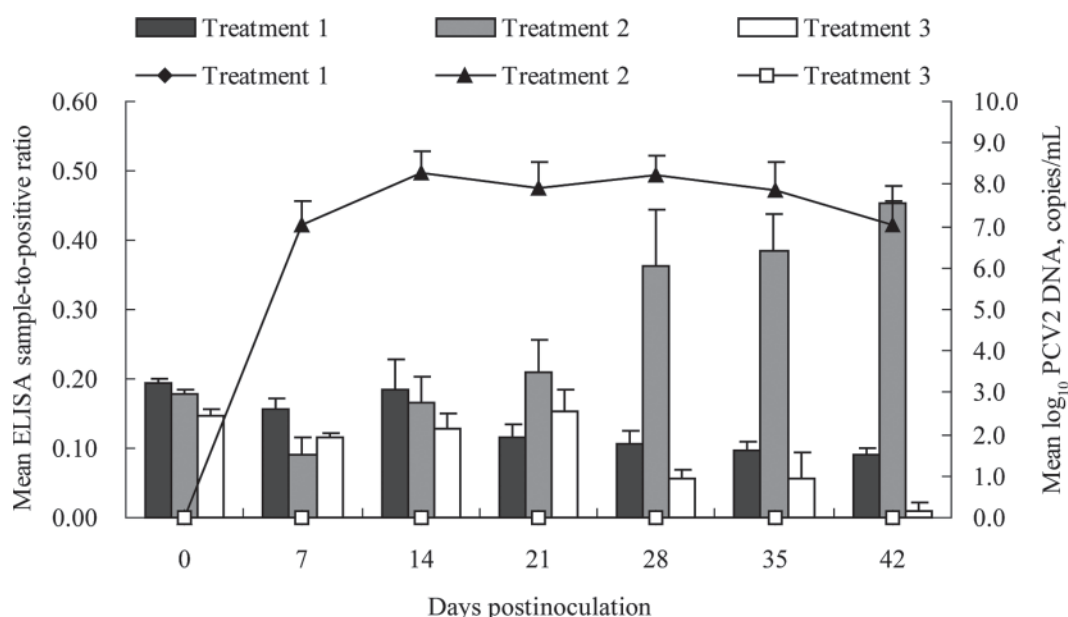


Figure 1. Porcine circovirus type 2 (PCV2) ELISA sample-to-positive ratios (bars) and amount of PCV2 DNA (lines) for treatment 1 pigs (negative control, no plasma in the feed, not inoculated with PCV2), treatment 2 pigs (positive control, no plasma in the feed, inoculated with PCV2), and treatment 3 pigs (4% porcine plasma in the feed for 42 d, not inoculated with PCV2). Error bars show the SEM in each group.

slaughter pigs is commonly included as an ingredient in nursery feeds (USDA, 2008). There are conflicting reports in the literature evaluating the potential for spray-dried porcine plasma included in swine feeds to be a vector for spreading PCV2. Pujols et al. (2008) reported that naïve pigs did not develop PCV2 viremia and had no evidence of seroconversion to PCV2 when fed a diet containing an increased quantity (8% plasma) of commercially produced porcine plasma that was PCR positive for PCV2 DNA ($5.4 \log_{10}$ copies/mL) for 45 d. In contrast, Patterson et al. (2010) reported that it is possible to transmit PCV2 to naïve pigs fed experimentally produced spray-dried porcine plasma collected from a single PCV2-infected pig exhibiting clinical symptoms of PCVAD. Thus, the current studies were done to investigate the prevalence of PCV2 DNA and antibody in commercially produced porcine plasma and the infectivity of PCV2 DNA-positive commercial spray-dried plasma fed to PCV2-naïve pigs.

The source animals for commercially produced porcine plasma are significantly different from the source animal reported by Patterson et al. (2010). Commercially available porcine plasma, including that used in the current study, is collected at federally inspected abattoirs from pigs that have passed antemortem inspection as fit for slaughter for human consumption, decreasing the chances for collection of plasma from a clinically affected animal. Typically, plasma is separated from the cellular fraction immediately after collection, chilled, and stored in insulated tanks. Daily, the liquid plasma is transported to the spray-drying facility in insulated tanker trucks containing plasma from approximately 9,000 to 10,000 pigs. At the spray-drying facility, the tanker load is stored in an insulated silo with 1 to 3 additional loads before further processing. Therefore, plasma from a single pig is pooled with that from 9,000 to 30,000 other pigs. Although a single pig may not have antibodies to all pathogens present in the population, pooling of such large numbers of animals results in a mixture likely to contain antibodies to all pathogens circulating in the population at any point in time (Borg et al., 2002). Pooling of plasma containing neutralizing antibodies with plasma containing pathogens such as viruses is recognized as a way to ensure neutralization of potentially contaminating viruses, especially stable viruses that are difficult to inactivate by other methods (Solheim et al., 2008; Williams and Khan, 2010).

During the time period when the commercially collected plasma was sampled, the incidence of PCV2 DNA was high (82.7%). The Midwest had a greater prevalence of PCV2 DNA in plasma samples collected from pigs slaughtered at the abattoirs compared with the Southeast. However, 100% of the samples contained anti-PCV2 antibodies. These data are consistent with serological surveys that have shown 100% of the investigated farms or individual pigs to be seropositive for PCV2 (Allan and Ellis, 2000; Walker et al., 2000; Zhou

et al., 2006). Processing fresh plasma at commercial spray-drying facilities had little effect from beginning to end on the presence or quantity of detectable PCV2 DNA or on detectable anti-PCV2 antibodies. Although the PCV2-neutralizing antibody was not measured in the spray-dried plasma samples, it has been reported that the quantity of PCV2-neutralizing antibodies coincides with the amount of antibodies detected by ELISA in PCVAD-free pigs (Meerts et al., 2006). In contrast, PCVAD-affected animals may have PCV2 antibodies, but neutralizing antibodies are very low or undetectable (Meerts et al., 2005, 2006). Therefore, considering the reduced PCV2 DNA in fresh, concentrated liquid, and spray-dried plasma samples and the health status of the source pigs at slaughter, it could be speculated that the final spray-dried plasma products contained PCV2-neutralizing antibodies that correlated with antibodies in the current study.

In the present animal trial, commercial spray-dried porcine plasma was selected from the available inventory based on the greatest amount of PCV2 DNA detected ($6.7 \log_{10}$ copies/g). The experimental design included a treatment in which the pigs were challenged directly with PCV2, demonstrating that the experimental pigs were susceptible to PCV2 infection. In this experiment, pigs inoculated with PCV2 (positive controls) became viremic by 7 dpi and remained viremic throughout the study. Additionally, all positive control pigs seroconverted to PCV2 by 42 dpi. However, at no time during the experiment did pigs consuming the diet containing spray-dried plasma become infected, as evidenced by the lack of PCV2 antigen in the tissues, lack of PCV2 viremia, or lack of PCV2 seroconversion. These data are consistent with those of Pujols et al. (2008). In contrast, Patterson et al. (2010) reported that spray-dried plasma collected from a single pig experimentally infected with PCV2 while exhibiting clinical symptoms of PCVAD was capable of transmitting PCV2. It is likely that differences in the source animals from which the inocula were produced contributed to the differences in results reported in these studies (Pujols et al., 2008; Patterson et al., 2010).

Spray drying is the transformation of a feed from a fluid state into a dried particulate by spraying the feed into a gaseous drying medium. The spray-drying process can be divided into 3 significant steps, including atomization of the liquid feed, interaction of the liquid droplet with the drying gases, and separation of the dried powder from the drying gases (Cal and Sollohub, 2010; Sollohub and Cal, 2010). The conditions in each step can affect the physical characteristics of the powder and microbial survival (Sollohub and Cal, 2010). For example, atomization can affect the droplet size, which in turn affects the rate of water evaporation from the particle and microbial survival (Prabakaran and Hoti, 2008; Thybo et al., 2008a). The angular momentum of a rotary atomizer affects shear forces during atomization and subsequent microbial survival

(Luna-Solano et al., 2005). The temperature of the drying gas affects the rate of evaporation and subsequent outlet temperature. The outlet temperature and rapid changes in temperature and pressure exert significant effects on microbial survival (To and Etzel, 1997a,b; Lian et al., 2002; Ananta et al., 2005). The design of the drying chamber affects the air flow and retention time of the particle within the drying chamber (Foster and Leatherman, 1995). The total time exposed to increased temperatures has been shown to affect microbial survival (APC Inc., Ankeny, IA, unpublished data) and is affected by the design of the system separating the dried particle from the heated air and transporting the dry powder to final packaging. Because the design and configuration of the spray-drying process have been shown to affect the effectiveness of spray drying, including microbial survival, it has been suggested that it may not be appropriate to fully extrapolate data from a laboratory spray drier to commercial spray drying (Thybo et al., 2008b). Spray drying has been shown to inactivate microbes (both bacterial and viral) in animal plasma. Pseudorabies virus ($10^{5.3}$ TCID₅₀/mL) and PRRSV ($10^{3.5}$ to $10^{4.0}$ TCID₅₀/mL) were both inactivated when added to bovine plasma and spray dried at an outlet temperature of 90°C, as evidenced by the absence of viable virus detected in 4 consecutive passages of cell cultures (Polo et al., 2005). Additionally, feeding 6-wk-old pigs a diet containing 8% spray-dried plasma for 63 d failed to elicit antibodies against parvovirus (a known virus model for heat-resistant viruses), PRRSV, or pseudorabies virus while improving the growth of pigs (Polo et al., 2005). However, PCV2 is resistant to heat inactivation (Welch et al., 2006). Freeze-dried PCV2 was shown to be resistant to dry-heat treatment at 120°C for 30 min (Welch et al., 2006). Other data demonstrated that the PCV2 could be completely inactivated after wet-heat treatment at 80°C for 15 min (O'Dea et al., 2008). It is likely that differences in the design and configuration of the spray-drying process between commercial production and a laboratory-scale drying system and the heat stability of PCV2 contributed to the contrasting results reported by Patterson et al. (2010) and Pujols et al. (2008), and the current study.

A greater serum PCV2 load has been correlated with an increased clinical severity of PCVAD in pigs (Brunborg et al., 2004; Krakowka et al., 2005; Segalés et al., 2005; McIntosh et al., 2009). It has been reported that PCVAD-affected pigs have a 3.0-log greater PCV2 load than nonaffected pigs (McIntosh et al., 2009), and 7.0 log₁₀ has been suggested as the threshold for PCVAD diagnosis (Olvera et al., 2004). In the present study, although an increased prevalence of PCV2 DNA (82.7%) was detected in fresh plasma samples collected directly from abattoirs, the relatively low amount of PCV2 DNA (5.2 ± 0.7 log₁₀; as-is basis) in the commercial spray-dried plasma suggested, as expected, that the source plasma was from subclinically infected pigs. The

reduced PCV2 DNA load found in commercial plasma, compared with the experimental plasma used by Patterson et al. (2010), may contribute to differences between studies in the literature and the current study.

Significant differences exist between the studies reported by Pujols et al. (2008), Patterson et al. (2010), and the current study. These differences include the health status of the source animals, the pooling of plasma from numerous animals, the design of the spray-drying equipment, and the PCV2 DNA load in the plasma. It is likely that a combination of these factors contributed to the differences in the reported results. In summary, the occurrence of PCV2 DNA and anti-PCV2 antibodies in spray-dried porcine plasma collected in the Midwest and East regions of the United States is widespread. However, the data from this study indicate that spray-dried porcine plasma as commercially collected and processed does not represent a significant risk factor for the transmission of PCV2 infection in commercial swine production when included in the ration as a feed ingredient.

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